In Vivo Efficacy of Gum Obtained *Pistacia Atlantica* in Experimental Treatment of Cutaneous Leishmaniasis

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Abstract

Background: Recent circumstantial evidences are suggesting that an increasing number of Iranian patients with cutaneous leishmaniasis are unresponsive to meglumine antimoniate (Glucantime®). *Pistacia atlantica* is native plant in Iran (central, western, and eastern regions). Gum obtained *Pistacia atlantica* has been reported to possess considerable *in vitro* antimicrobial activity. In this study, we aimed to investigate antileishmanial activity of *P. atlantica*.

Methods: Male BALB/c mice were inoculated subcutaneously 2×10^6 *L. major* Promastigotes (MHROM/IR/75/ER) at the base of tail in 2007. Mice were randomly divided into 3 groups. in group 1 Glucantime® was administered to the BALB/c mice in regimen of 60 mg per kg of body weight for 28 days by intraperitoneal injections per day, in group 2 the gum of *P. atlantica* var. *Kurdica* were tested by rubbing of local lesions for 28 days, group 3 infected but non-treated. Comparisons of treated groups and untreated group were done by two-way analysis of variance (ANOVA)

Results: Topically rubbing administration of gum obtained *P. atlantica* var. *kurdica* daily for 28 days like Glucantime® decreased skin lesion size in the BALB/c mice infected with *L.major* compared with that in the control ($P< 0.01$). Treatment BALB/c mice with gum obtained *P. atlantica* var. *kurdica* and Glucantime® causes decrease number of parasitologicaly positive mice ($P< 0.05$).

Conclusion: Our results show that gum obtained *P. atlantica* var. *kurdica* can be used for controlling cutaneous leishmaniasis caused by *L.major* and inhibiting development of cutaneous leishmaniasis lesions.

Keywords: *Cutaneous leishmaniasis, Pistacia atlantica,* L. major*, Balb/c*

Introduction

Cutaneous leishmanisis (CL) is a disease resulting from infection with species of the genus *Leishmania* including *L. major* and *L. tropica* in different parts of Iran (1, 2). *L. major* causes zoonotic cutaneous leishmaniasis (ZCL) in many rural areas of Iran (3). CL can be associated with significant morbidity and occasional deforming scars. Pentavalent antimonial compounds (sodium stibogluconate and meglumine antimoniate) have been the first drugs of choice in last decades for the treatment of this disorder. These drugs are parenteral and associated with significant side effects (4). These compounds, particu-

larly meglumine antimoniate, are the first line drugs for the treatment of all forms of leishmaniasis in Iran too (5, 6). Based on a few studies that have been carried out in recent years, about 10 to 15% of CL has not desirable response to meglumine antimoniate in Iran (7). Recent circumstantial evidences are suggesting that an increasing number of Iranian patients with cutaneous leishmaniasis are unresponsive to meglumine antimoniate (Glucantime®) (7). Based on a recent studies miltefosine as an oral drug is apparently at least as good as meglumine antimoniate for the treatment of cutaneous leishmaniasis caused by *L. major* in Iran (6, 8).

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The genus *Pistacia* belongs to the family Anacardiaceae. Among 15 known species of pistachios, only 3 species grow in Iran, including *P. vera, P. Khinjuk* and *P. atlantica*. They are the most important species of *pistachio* and for this reason, Iran is known as the origin of *pistachios* (9). *P. atlantica* is native to a number of countries of temperate Asia, e.g. Armenia, Azerbaijan, Iran, Iraq, Turkey, etc (10). In Iran, this plant grows in the central, western, and eastern areas (11). The resin of this plant, mastic gum, is obtained as an exudate after hurting the trunk and branches. Mastic gum has been used in traditional Greek medicine for various gastrointestinal disorders like gastralgia, dyspepsia and peptic ulcer for more than 2500 years. Ancient Greek physicians, such as Hippocrates, Dioscorides, Theophrastos and Galenos mentioned its properties and recommended its use (12). Mastic gum has been reported to possess considerable *in vitro* antibacterial and antifungal activity (13). Total mastic extract without polymer might be effective in reducing *Helicobacter pylori* colonization (12).

Because of existing high percentage of an insoluble and sticky polymer (poly-B-myrcene) in gum that hinders its oral administration and reduces bioavailability and that *L. major* causes topical lesion, in this study we aimed to determine cure rate of topical administration of *P. atlantica* gum on BALB/c mice infected by Iranian strain of *L. major* (MRHO/IR/75/ER).

Materials and Methods

Animal infection
Male BALB/c mice, 6-8 wk old with a body weight of approximately 20 g, used in this study. The animals were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran,Hesarak, Karaj, Iran. Male BALB/c mice were inoculated subcutaneously about 2×10^6 *L. major* Promastigotes (MHROM/IR/

75/ER) at the base of tail. Disease progression was monitored by parasitological examination of lesions after 4 to 8 wk post inoculation.

Ethical consideration
This study was conducted in adherence to ethical standards required for animal subjects protection.

Parasites
Iranian reference strain *L. major* promastigotes (MHROM/IR/75/ER) (prepared in the Protozoology Unit of the School of Public Health, Tehran University of Medical Sciences, Iran) were grown in RPMI-1640 supplemented with 15% inactivated fetal calf serum (FCS), 100 mg/ml streptomycin and 100 IU/ml penicillin G at 23-25 °C. Promastigotes from stationary-growth phase cultures were used for infection of mice.

Gum and Glucantime® treatment and evaluation
The gum of *P. atlantica* var. *kurdica* was collected from Kerend of Kermanshah (western part of Iran) summer 2007. The gum was obtained as an exudate of the trunk and branches. Glucantime® (Rorer Rhone-Poulenc Specia, Paris, France) kindly received from the Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences. Treatment for both substances was initiated after inoculation, when the infection was well preformed and local lesions were obvious. Before treatment diameter of lesions were measured and the mice were randomly divided into 3 groups of 15. in group 1 Glucantime® was administered to the BALB/c mice in regimen of 60 mg per kg of body weight for 28 d by intraperitoneal injections per day, in group 2 the gum of *P. atlantica* var. *Kurdica* were tested by daily rubbing of local lesions for 28 d, group 3 infected but non-treated.

Before treatment, four and 8 wk after beginning treatment, diameter of skin lesions

were measured and impression smears were prepared from them lesions, the slide were fixed with absolute methanol, stained with Geimsa stain10% and examined by light microscopy with high magnification (X1000). Treatment effects of gum were determined by compared to Glucantime®and non-treatment groups by measuring the size of the skin lesions and the number of parasitologicaly positive and negative mice in treated with gum and Glucantime® and control.

Statistical analysis

The mean and standard deviation were calculated by using Microsoft EXCEL soft ware. Comparisons of treated groups and untreated group were done by two-way analysis of variance (ANOVA). Data were considered statistically significant at $P< 0.05$.

Results

The antileishmanial activity of the gum obtained *P. atlantica var. kurdica* was first tested in vivo on *L. major*. Gum of *P. atlantica* exhibited growth-inhibitory activity on *L. major* comparison control.

The lesions diameter in different groups of mice was measured before treatment, four and 8 wk after treatment. Table 1 shows that topically rubbing administration of gum obtained *P. atlantica* var. *kurdica* daily for 28 d prevented lesion development in the BALB/c mice infected with *L. major* compared with that in the control.

Effect of treatment of BALB/c mice infected with *L. major* with Glucatime at dose of 60 mg/kg daily for 28 d by the intraperitoneal route is presented in Table1.data in this table show decreasing size of lesion comparison control ($P< 0.01$).

The parasite existence in leishmanial lesions were examined in three groups of mice before treatment and four and 8 wk after treatment. Table 2 presents the results of the effect of gum obtained *P. atlantica* var. *kurdica* and Glucantime® on parasite existence in the BALB/c mice infected with *L. major* before treatment, four and 8 wk after treatment comparison that in the control animals.

As shown in Fig. 1, a decrease in the lesions size is observed in mice treated with gum obtained *P. atlantica* var. *kurdica* and Glucantime® comparison control animals ($P< 0.01$). Statistical analysis of Table 1 data shows treatment with gum obtained *P. atlantica* var. *kurdica* and Glucantime® causes decrease number parasitologicaly positive mice ($P< 0.05$).

Table 1: Inhibitory effects of treatments Lesion size reduction (mm) with Glucantime® and gum obtained *Pistacia atlantica* var. *kurdica* on leishmanial skin lesion sizes in BALB/c mice infected with *L.major*

	Week after treatment		
Groups	**0**	**4**	**8**
Glucantime®	67.01±12.46	36.14±5.01	31.9±8.02
gum*	68.4±6.92	40.36±4.98	35.3±5.01
Control	73.8±8.01	86.08±7.87	88.01±7.68

*gum of *pistacia atlantica* var. *kurdica*

Table 2: Effect of treatment with Glucantime® and gum obtained *Pistacia atlantica* var. *kurdica* on parasite existence in BALB/c mice infected with *L.major*

	Week after treatment					
	0		**4**		**8**	
substance	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**
Glucantime®	0	15	8	7	8	7
gum*	0	15	5	10	5	10
Control	0	15	0	15	0	15

*gum of *pistacia atlantica* var. *kurdica*
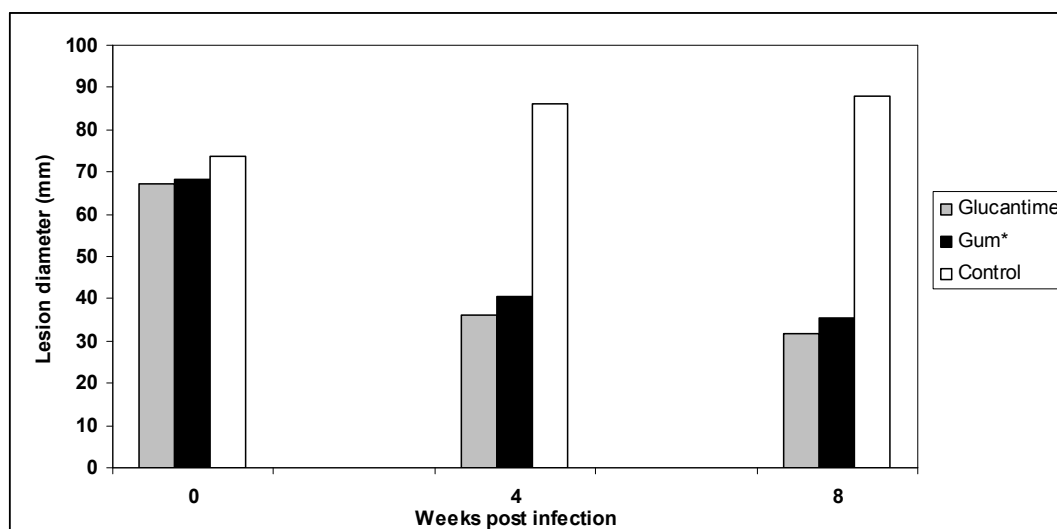
Fig. 1: Treatment effects of Glucantime® and gum obtained *Pistacia atlantica* var. *kurdica* on leishmanial lesions sizes (mm) in BALB/c mice infected with *L.major*

Discussion

Despite the tremendous progress made in the understanding of the molecular biology of *Leishmania* and the clinical possibilities presented by some experimental chemothera-peutic agents, a few drugs have been developed for treating leishmaniasis (14). Also development of a new drug for the treatment of human leishmaniasis has been impeded by the lack of a simple, rapid drug-evaluation system that is universally applicable to the various *Leishmania* species (15).

A range of treatment options exists in leish-maniasis, which include two pentavalent an-timonails, amphoterisin B, paromomycin, pen-tamidine, and the new oral agent miltefosine (16, 17).

Most of the commonly used antileishmanial drugs, such as pentavalent antimonail agents, exhibit considerable toxicity, and there are reports of large-scale clinical drug resistance among the organisms visceral leishmaniasis (18). The second- line drugs, such as ampho-terisin B and pentamidine, do not have a therapeutic index as well as that of SbV, long-term therapy is often required, and they have toxic effects (19). About 75% of reported CL cases from Iran are zoonotic cutaneous leish-maniasis caused by *L. major* (3).

The crude gum of *Pistacia* contains an in-soluble polymer (poly-B-myrcene), monoter-penes (such as α-pinene, limonene, α- phel-landrene, β-pinene, β-myrcene, 3-carene, al-dehyde citral, epoxypinene, limonene oxide) and triterpenes (oleanonic acid, moronic acid, 24Z-masticadienonic acid, 24Z-isomas-ticadienonic acid, 24Z-masticadienolic acid, and 24Z-isomasticadienolic acid) (11, 12).

Some studies have shown that terpenic com-pounds such as labdone diterpene (isolated from *Polyalthia macropoda*), the phorbol ester, iridoid glycosides (found in *Picrorhiza ku*rroa and *Nyctanthes arbortritis*) and Picroliv (isolated from *Picroliv kurroa*) have antileishmanial effects (20-22).

Our results show that gum obtained *P. at-lantica* var. *kurdica* can used for controlling cutaneous leishmaniasis caused by *L. major*, to inhibit development of cutaneous leishma-niasis lesions(*P*< 0.01) and to be active against *L. major* in 30% of mice (*P*< 0.05).

With regard to the sticky polymer in gum re-duces bioavailability of the contained active compounds, our results suggest if full ad-

sorption of active compounds the effect of gum will increase.

Recently, the resistance against antimonial drugs, toxicity and side effects of systemically administrative drugs have been reported (6, 7, 18), therefore there is a great for the development of effective, safe and topically administrative drugs for the different forms of leishmaniasis.

Acknowledgements

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